

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Marcus A. Horwitz : Group Art Unit 186  
LEGIONELLOSIS VACCINES : Examiner: Mohamed, A.  
AND METHODS FOR THEIR  
PRODUCTION

Serial No. 232,664

Filed: August 16, 1988

: Docket No.: 70-155

DECLARATION UNDER RULE 132

Honorable Commissioner of Patents  
and Trademarks  
Washington, D.C. 20231

Sir:

I, Marcus A. Horwitz, M.D., declare and state that:

1. I am the inventor in the above-identified patent application.

2. I received my M. D. Degree from Columbia University College of Physicians and Surgeons, New York, New York in June of 1972 and am currently Professor of Medicine and of Microbiology and Immunology, Chief, Division of Infectious Diseases, Department of Medicine, UCLA School of Medicine, Center for the Health Sciences at Los Angeles, California. A copy of my current curriculum Vitae

detailling my Education, Internship and Residency, Public Health Service Positions, Clinical Fellowships, Research Fellowships, Faculty Positions, Certifications, Affiliations, Honors and Awards, Scientific and Editorial Boards and Study Sections, Publications, Abstracts, and Presentations at National or International Meetings was previously made of record as Exhibit 1 to my earlier Declaration Under Rule 132 filed June 4, 1990. As indicated in my Curriculum Vitae, I have extensive experience in the fields of Microbiology, Immunology and Infectious Diseases.

3. I have reviewed the Official Action dated August 9, 1991, wherein the Examiner requested that I provide my actual data showing that Mycobacterium tuberculosis extracellular proteins induce cell-mediated immunity in humans. Accordingly, I have tabulated this data in the attached Tables I and II and plotted the data from both tables in the attached Figure 1. The protocol for the generation of this data is as follows.

4. To determine if M. tuberculosis extracellular proteins induce cell-mediated immunity in humans, we studied the proliferative responses to M. tuberculosis extracellular proteins of lymphocytes from persons previously infected with M. tuberculosis, as evidenced by a positive skin test response to purified protein derivative (PPD) of M. tuberculosis (PPD+ persons) and lymphocytes from persons not previously infected with M.

tuberculosis, as evidenced by a negative skin test response to PPD (PPD- persons).

5. In a standard lymphocyte proliferation assay, lymphocytes from PPD+ persons responded strongly to Extracellular Proteins of M. tuberculosis including the Major Extracellular Protein. A representative experiment is shown in Table I, Experiment 1 in which there was a peak stimulation index of 29.9 in response to Extracellular Proteins and a peak stimulation index of 38.6 in response to the Major Extracellular Protein.

6. In contrast, lymphocytes from PPD- persons responded weakly, if at all, to M. tuberculosis extracellular proteins. A representative experiment is shown in Table 1, Experiment 2 in which there was a peak stimulation index of only 5.0 (day 4) in response to Extracellular Proteins and 4.5 (day 2) in response to the Major Extracellular Protein.

7. Cumulative results from 8 independent experiments on 3 PPD+ and 3 PPD- persons are tabulated in Table II. Peak stimulation indices for lymphocytes from PPD+ persons in response to M. tuberculosis Extracellular Proteins averaged  $47.6 \pm 12.9$  (Mean + S.E.), whereas peak stimulation indices for lymphocytes from PPD- persons in response to M. tuberculosis extracellular proteins averaged  $7.5 \pm 4.5$  (Mean +S.E.).

8. Thus, the mean response of lymphocytes from PPD+ persons

was over 6-fold that of lymphocytes from PPD- persons to M. tuberculosis extracellular proteins. Peak stimulation indices for lymphocytes from PPD+ persons in response to M. tuberculosis Major Extracellular Protein averaged  $30.2 \pm 5.8$  (Mean + S.E.), whereas peak stimulation indices for lymphocytes from PPD- persons in response to M. tuberculosis Major Extracellular Protein averaged  $2.7 \pm 0.9$  (Mean + S.E.). The mean response of lymphocytes from PPD+ persons was over 11-fold that of lymphocytes from PPD- persons to M. tuberculosis Major Extracellular Protein.

9. The striking differences in the magnitude of the proliferative responses to M. tuberculosis Extracellular Proteins of lymphocytes from PPD+ and PPD- persons can be further appreciated from Figure 1 in which data from Table II is plotted. These data clearly demonstrate that persons infected with M. tuberculosis develop a very strong cell-mediated immune response to M. tuberculosis extracellular proteins.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine, or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize

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the validity of the above-identified patent application or any patent issuing thereon.

Date: 10/30/91

Marcus A. Horwitz  
Marcus A. Horwitz, M.D.

TABLE I

TWO REPRESENTATIVE EXPERIMENTS DEMONSTRATING THAT PERIPHERAL BLOOD LYMPHOCYTES FROM PPD+ PERSONS BUT NOT PPD- PERSONS PROLIFERATE MARKEDLY TO EXTRACELLULAR PROTEINS OF M. TUBERCULOSIS AND THE MAJOR EXTRACELLULAR PROTEIN

I. Lymphocyte Proliferation to Extracellular Proteins of M. tuberculosis:

Expt	Patient	PPD	Status	Day	Stimulation Indices* in Response to Indicated Amount of Extracellular Protein(ug/ml)			
					0.01	0.1	1.0	10.0
1	DG	+	+	4	2.4	15.7	27.3	29.9
2	DK	-	-	2	0.2	0.6	1.4	1.6
				4	0.5	1.1	3.4	5.0

II. Lymphocyte Proliferation to Major Extracellular Protein of M. tuberculosis

Expt	Patient	PPD	Status	Day	Stimulation Indices* in Response to Indicated Amount of Major Extracellular Protein(ug/ml)			
					0.01	0.1	1.0	10.0
1	DG	+	+	4	3.9	12.7	38.6	35.2
2	DK	-	-	2	0.4	1.9	4.5	3.8
				4	1.0	2.0	3.9	1.1

Peripheral blood lymphocytes were purified by conventional methodology from a person who was PPD+ (Experiment 1) and a person who was PPD- (Experiment 2). The lymphocytes were incubated in microtest wells at 37°C in 5% CO<sub>2</sub>- 95% air for 2 or 4 days in tissue culture medium containing autologous serum, polymixin B, and 0, 0.01, 0.1, 1.0, or 10 ug/ml M. tuberculosis extracellular proteins or M. tuberculosis Major Extracellular Protein. The lymphocytes were then assayed for their capacity to incorporate <sup>3</sup>H-thymidine, and Stimulation Indices were calculated.

(mean <sup>3</sup>H-thymidine incorporation of lymphocytes incubated with antigen)

\*Stimulation Index=-----

(mean <sup>3</sup>H-thymidine incorporation of lymphocytes incubated without antigen)

Table II

PERIPHERAL BLOOD LYMPHOCYTES FROM PPD+ BUT NOT PPD- PERSONS  
PROLIFERATE MARKEDLY IN RESPONSE M. TUBERCULOSIS  
EXTRACELLULAR PROTEINS

I. Lymphocyte Proliferation to Extracellular Proteins  
of M. tuberculosis

<u>PPD+</u> Persons		<u>PPD-</u> Persons	
Expt.	Peak <u>SI</u> *	Expt.	Peak <u>SI</u> *
B	24.5	F	5.0
C	29.9	G	16.3
D	55.0	H	1.1
E	80.9		

Mean+SE:      47.6+12.9                          7.5+4.5

II. Lymphocyte Proliferation to Major Extracellular Protein  
of M. tuberculosis

<u>PPD+</u> Persons		<u>PPD-</u> Persons	
Expt.	Peak <u>SI</u> *	Expt.	Peak <u>SI</u> *
A	19.2	F	4.5
B	18.1	G	2.2
C	38.6	H	1.4
D	48.3		
E	26.7		

Mean+SE:      30.2+5.8                                  2.7+0.9

Peripheral blood lymphocytes were purified by conventional methodology from 3 PPD+ and 3 PPD- persons and assayed in 8 independent experiments. The lymphocytes were incubated in microtest wells at 37°C in 5% CO<sub>2</sub> - 95% air for 2-4 days in tissue culture medium containing autologous serum, polymixin B, and 0, 0.01, 0.1, 1.0, or 10.0 ug/ml M. tuberculosis Extracellular Proteins or M. tuberculosis Major Extracellular Protein. The lymphocytes were assayed for their capacity to incorporate <sup>3</sup>H-thymidine, and Stimulation Indices were calculated. Values are the peak Stimulation Index for each experiment.

(mean <sup>3</sup>H- thymidine incorporation to  
lymphocytes incubated with antigen)

\*Stimulation Index=-----

(mean <sup>3</sup>H-thymidine incorporation of  
lymphocytes incubated without antigen)

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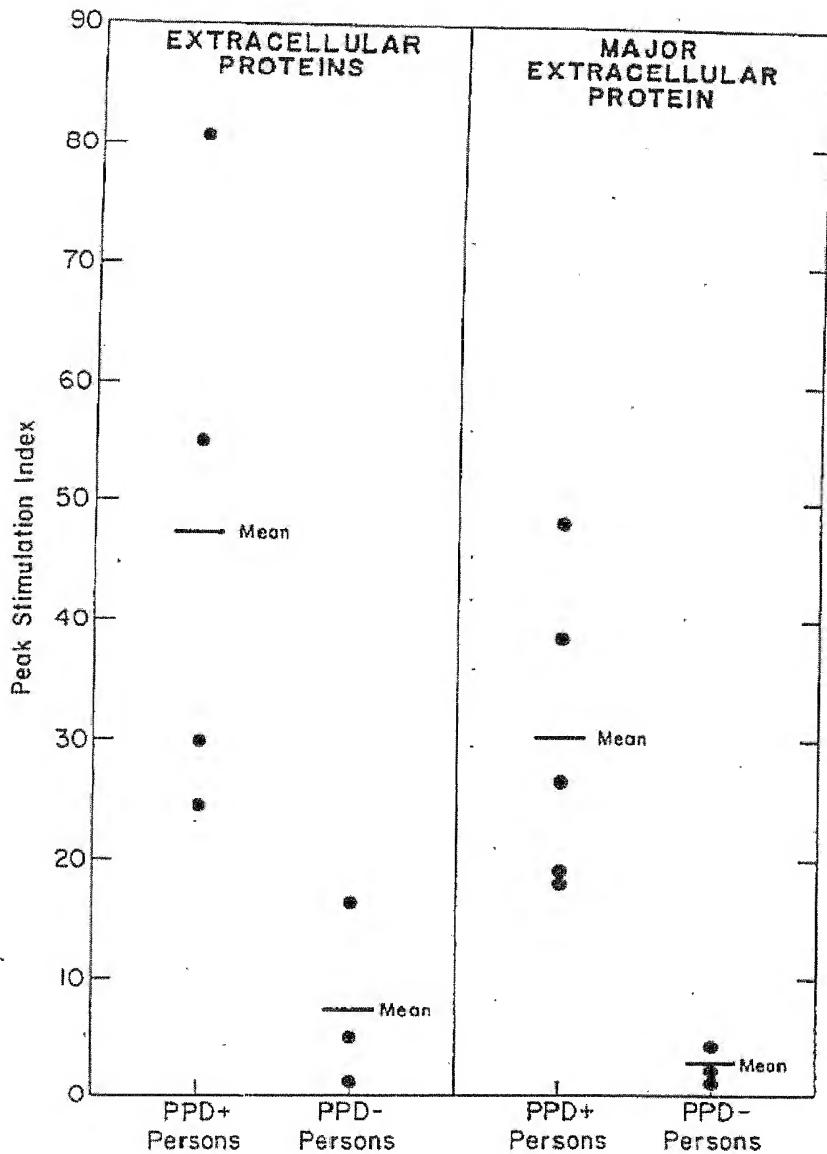


Figure 1. Peripheral blood lymphocytes from PPD+ but not PPD- persons proliferate markedly in response to M. tuberculosis Extracellular Proteins. Peak stimulation indices (data from Table II) of PPD+ and PPD- persons in response to Extracellular Proteins of M. tuberculosis or the Major Extracellular Protein of M. tuberculosis (right) are graphed.

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## CURRICULUM VITAE

### Peter Andersen

- 2006 - Honorary Professor, The Royal Veterinary and Agricultural University, Department of Veterinary Pathobiology
- 2002- Vice President, Vaccine R & D, Director, Department of Infectious Disease Immunology
- 1997- 2002 Head of Department of TB Immunology
- 1996 D.Sc., Copenhagen University
- 1994 Head of the Tuberculosis Research Unit at Statens Serum Institut
- 1993: Position as senior scientist at the Bacterial Vaccine Department, Statens Serum Institut
- 1991-1993 Co-ordinator of the WHO collaborative research project: "The search for antigens of immunological relevance from *M. tuberculosis*"
- 1991-1992 Recipient of a fellowship from the Danish Association against Lung Diseases
- 1989-1991 Postgraduate scholarships from the Royal Veterinary and Agricultural University
- 1989 Scholarships at the Laboratory of Cellular Physiology, Rockefeller University, New York (Dr. Kaplan)
- 1988-1989 Research fellow at the Vaccine Department, Statens Serum Institut
- 1988 DVM from the Royal Veterinary and Agricultural University
- 1986-1987 Scholarships at the Marine Laboratory, Aberdeen, Scotland (Dr. Ellis) and the Marine Laboratory, Hokkaido, Japan (Dr. Sakai)

Peter Andersen, DVM, D.Sc, born 7 December 1961. As Vice President of Vaccine Research and Development at Statens Serum Institut, as well as co-ordinator of a number of International Research Grants, he has had extensive experience in assembling and directing multi-disciplinary research teams. Prof. Andersen's research has been focused on the identification and characterisation of antigens, immune mechanisms and vaccine delivery systems that mediate protection against important pathogens such as *Mycobacterium tuberculosis* and *Clamydia trachomatis*. In his current position, Prof. Andersen is responsible for the overall coordination of vaccine research and development at the SSI, covering activities from early research and to clinical development with more than 80 employees. This program currently has 2 different TB a novel liposomal adjuvant formulation and a new TB skintest under clinical testing and a number of experimental vaccines in the late preclinical stage. In collaboration with industrial partners the SSI antigen discovery programmes has furthermore resulted in three commercially available tests for TB diagnosis.

Dr. Andersen has served on a number of committees to advise and co-ordinate strategies for vaccine and diagnostic development. He has been organising and chairing several

at scientific meetings in the last eight years. Dr. Andersen is the inventor of more than 20 patent families and has published 200 papers within the field of infection, immunity and vaccine research in peer-reviewed journals.

**Scientific committees:**

- Steering Committee for TBVI "Tuberculosis Vaccine Initiative ". (2007-)
- Organizing Committee for The First Global Symposium on Interferon- $\gamma$  Assays "Rethinking the natural history and epidemiology of tuberculosis infection" (2006-7)
- Steering Committee for MUVAPRED "Mucosal Vaccines for Poverty Related Diseases" (2005- )
- Head of SSI-Centre of Vaccinology (2004- )
- Steering Committee for TBVAC "An integrated project for the design and testing of vaccine candidates against tuberculosis; identification, development and clinical studies". (2004-)
- Technical Advisory Group for the Foundation for Innovative New Diagnostics (FIND) (2004)
- Steering Committee for WHO's Initiative for Vaccine Research (IVR) (2004-)
- Member of Advisory Group for the NIH, NIAID Contract NO1 AI-75320, "TB Research Materials and Vaccine Testing" (2002-)
- Section Editor for the journal Tuberculosis (2002-)
- Steering Committee for TB VACCINES FOR THE WORLD CONFERENCE - TBV (2003, 2006 and 2008)
- Steering Committee for WHO's Tropical Disease Research (TDR) (2001-)
- Steering group for WHO's Initiative for Vaccine Research (IVR) (2001-)
- High-Level Scientific Conferences Panel, European Commission (2001)
- Chair on Global Forum on TB Vaccines Research and Development, WHO (2001)
- Member of the management team for the European TB Vaccine Cluster (2000-2004)
- Member of the editorial board of Infection and Immunity (1995-1999)
- WHO adviser on TB Vaccine development (Immyc) (1993-1998)
- Chair of the meeting on TB vaccines organised by the International Union against TB and Lung Diseases (IUATLD) (1998)
- Organising committee of the scientific meeting "Cellular mechanism and molecules in Infection and Immunity" (1999 + 2002)
- Chairman of the WHO organised animal model task force for the evaluation of experimental tuberculosis vaccines (1998)
- Organising committee of The International Symposium on Tuberculosis Vaccine Development and Evaluation, San Francisco (1998)
- EU cost/STD initiated expert panel on vaccines against TB (1996)
- Committee member of The Elsinore Meeting on Infection Immunity (1993-)

**Prizes and awards:**

2006	Honorary Professor, The Royal Veterinary and Agricultural University, Department of Veterinary Pathobiology
2005	Thomson Scientific's distinction as the most quoted Danish scientist over the last 15 years in the field of Immunology
2004	Professor, dr.med. Fritz Kaufmann Memorial Fund
1999	Thorvald Madsen Research Fund in recognition of an outstanding research achievement

**Patents:**

- New Fusion proteins (PCT/DK2006/000356)
- "Subdominant epitopes" PA 2006 00861
- "CFP7 and homologues thereof" (Continuation in part, Plougmann, Vingtoft & Partners ref. No. 20486 US 04)
- "A new specific epitope based immunological diagnosis of tuberculosis" 34545/PCT/DK2004/00314
- "Chlamydia trachomatis antigens and their use" PA 2004 01633
- "Malaria Vaccine" WO2004043488
- "Compositions and methods for stabilizing lipid based adjuvant formulations using glycolipids" PA 2004 01070
- "Improved Tuberculosis Vaccines" 36109DK1
- "Freeze-dried Vaccine Adjuvant" 15012/PA 2003 01920
- "Adjuvant combinations of liposomes and mycobacterial lipids for immunization compositions and vaccines" 15010/PA 2003 01046
- "ESAT-6-Ag85B hybrid" (Continuation in part, Plougmann, Vingtoft & Partners ref. No. 20486 US 03)
- "Tuberculosis vaccine and diagnostic based on the *Mycobacterium tuberculosis* ESAT-6 gene family" (PCT/DK00/000039, US application 60/144,011, WO0104151)
- "TB vaccine and diagnostic based on antigens from the *Mycobacterium tuberculosis* cell" (PCT/DK99/00538, US application 09/415,884, WO021983)
- "Nucleic acid fragments and polypeptide fragments derived from *Mycobacterium tuberculosis*" II (PCT/DK98/00438, WO99/24577, US patent application 09/246,191)
- "Nucleic acid fragments and polypeptide fragments derived from *Mycobacterium tuberculosis*" I (PCT/DK98/00132, WO98/44119, US patent application 09/050,739)
- "New Diagnosis skin test for Tuberculosis" (PCT/DK94/00270, WO95/01440, US patent application 08/569,221)
- "A polynucleotide functionally coding for the lhp protein from *Mycobacterium tuberculosis*, its biologically active derivative fragments, as well as methods using the same" (PCT/IB98/01091)
- "Adjuvant combinations for immunization composition and vaccines" (US patent application 09/310,551)
- "Tuberculosis vaccine" (PCT/DK01/00079, WO02/01441, US patent 6,028,077)

### **International grants:**

- Gates Foundation; Grand Challenge in Global Health (GCGH). "Biomarkers of protective immunity and surrogate markers of TB disease in Africa " (2005-2009)
- Gates Foundation; Grand Challenge in Global Health (GCGH). "Preclinical Evaluation of Post-Exposure TB Vaccine" (2005-2009) (8 partner program coordinated by P. Andersen)
- AERAS Global TB Vaccine Foundation, Research Collaboration Agreement (2005-2007)
- EDCTP "Studies of surrogate markers of drug efficacy, disease activity and relapse in tuberculosis." EDCTP Code 2004.01.T.d1 (2005-2007)
- EU project, Contract LSHP-CT-2003-503367, TBVAC "An integrated project for the design and testing of vaccine candidates against tuberculosis: identification, development and clinical studies". (2004-2008)
- EU project, Contract LSHP-CT-2003-503240, MUVAPE "Mucosal Vaccines for Poverty Related Diseases" (2004-2008)
- WHO, "Vaccination against Latent Tuberculosis" (2003-2004)
- EU project, Contract ICA4-CT-2002-10052, VACSIS "Host-parasite relationship in susceptibility to tuberculosis" (2002-2005)
- EU project, Contract ICA4-CT-1999-10005; INCO-DEV funding for VACSEL project "Longitudinal human study on development of tuberculosis" (1999-2003)
- NIH project, Contract N01-AI-95383, TBRU consortium application. (1999-2002)
- EU project, Contract QLG2- CT- 1999- 00660: "A functional genomics study of lysyl-tRNA synthesis as a target for the diagnosis and treatment of microbial infections and mitochondrial myopathies" (1999- 2003)
- EU project, Contract ICA4-CT-2000-30023: "Identification of relevant diagnostic antigens for bovine tuberculosis: influences of animal and regional disease patterns" (2000-2003)
- EU project, Contract QLRT-2001-02018: "Structural and functional genomics of *Mycobacterium tuberculosis*" (2002-2004)
- EU project, Contract QLRT-2001-01702: "Novel approaches to induce mucosal immunity against TB using the combined adjuvant strategy of CTA1-DD and ISCOMS" (2001-2004)
- EU project, Contract QLK2-CT-1999-01093 TB Vaccine Cluster (2000-2003)
- EU project (INCO-DEV): Identification of relevant diagnostic antigens for bovine tuberculosis: Influences of animal and regional disease patterns (2000-)
- Partner in the EU programmes "New *Mycobacterium tuberculosis* antigens for diagnosis and vaccines" and "Development of novel vaccines by attenuation of *M. tuberculosis*", co-ordinated by Institut Pasteur (1997-2000)
- EU project, Contract IC18-CT97-0254: "Development of tuberculosis vaccine with consistent efficacy in different regions of the world" (1997-2000)
- EEC STD-3 project, Contract TS3\*CT94-0313: "Development of an improved vaccine against *Mycobacterium tuberculosis*" (1994 - 97)
- WHO, Global Vaccine Programme, "The search for antigens of immunological relevance from *M. tuberculosis*" (1992-97)

**Doctor of Science opponent**

Antigens of *Mycobacterium avium* subspecies *paratuberculosis*, I. Olsen, Oslo, Norway 2001

**PhD. opponent**

Augmentation of adenovirus induced immune responses Evaluation of administration and antigen presentation with respect to the DC8+ T cell mediated immune response induced by replication defective adenovirus, P. J. Holst, Copenhagen, Denmark, 2008

Cellular Immune Responses during Latent Tuberculosis, E. M. S. Leyten, The Hague, The Netherlands, 2008

Antiviral Protection by Monospecific CD8 T Cells Primed through DNA Immunization, C. Bartholdy, Copenhagen, Denmark, 2003

Cytokines as potential co-adjuvants in a subunit vaccine against tuberculosis, I. S. Leal, Porto, Portugal, 2001

The interplay of cytokines and chemokines in virus-induced T-cell mediated inflammation, A. Nansen, Copenhagen, Denmark, 2000

Identification of novel *Leishmania* antigens and development of a DNA vaccine against leishmaniasis, A.T.R. Jensen, Copenhagen, Denmark 2000

**MSc assessments**

The pathogenesis of experimental malaria (thesis in Danish), N.S. Sørensen, Copenhagen Denmark, 1996

Investigation of the human immune response to antigens isolated from actively-growing *Mycobacterium tuberculosis* (thesis in Danish), H. T. Boesen, Copenhagen, Denmark 1994

**PhD. supervision**

On the mechanisms of selected adjuvants, K.K. Smith, Copenhagen, Denmark, 2007

Identification of Markers of Apoptosis as Correlates of Protection or Susceptibility in Tuberculosis, M. A. Alemayehu, Copenhagen, Denmark 2007

Antigenic Profiling of *Chlamydia trachomatis*, F. Follmann, Copenhagen, Denmark, 2007

Pre-clinical evaluation and characterisation of adjuvant formulation in novel subunit vaccines against *Mycobacterium tuberculosis*, EA Agger, Copenhagen Denmark, 2006

Immune recognition of novel antigens from *M. tuberculosis* with potential vaccine and diagnostic utility, A. Demissie Area, Copenhagen, Denmark, 2004

Vaccination against tuberculosis with the mycobacterial antigen, ESAT-6 in naïve and sensitised mice, L. Brändt, Copenhagen, Denmark, 2000

Identification, purification and characterization of *Mycobacterium tuberculosis* culture filtrate proteins, K. Weldingh, Copenhagen Denmark, 1999

Immunity against *Mycobacterium Tuberculosis* in Humans, P. Ravn, Copenhagen, Denmark 1996

**MSc supervision**

An immunological Investigation of the DDA/TDB-Adjuvant, K.V. Knudsen, Copenhagen, Denmark 2002

Immunological characterisation of subcellar fractions from *Mycobacterium tuberculosis*, E.A. Agger, Copenhagen, 1998

## Publications

1. Lillebaek T, Bergstedt W, Tingskov PN, Thierry-Carstensen B, Aggerbeck H, Hoff ST, Weldingh K, **Andersen P**, Soeborg B, Thomsen VO, Andersen AB. Risk of sensitization in healthy adults following repeated administration of rESAT-6 skin test reagent by the Mantoux injection technique. *Tuberculosis (In Press)*
2. Werninghaus K, Babiak A, Gro O, Hlscher C, Dietrich H, Agger EM, Mages J, Mocsai A, Schoenen H, Finger K, Nimmerjahn F, Brown GD, Kirschning C, Heit A, **Andersen P**, Wagner H, Ruland J, Lang R. Adjuvanticity of a synthetic cord factor analogue for subunit *Mycobacterium tuberculosis* vaccination requires FcR $\{\gamma\}$ -Syk-Card9-dependent innate immune activation. *J Exp Med.* 2009 Jan 16;206(1):89-97
3. Morera Y, Bequet-Romero M, Ayala M, Lamdán H, Agger EM, **Andersen P**, Gavilondo JV. Anti-tumoral effect of active immunotherapy in C57BL/6 mice using a recombinant human VEGF protein as antigen and three chemically unrelated adjuvants. *Angiogenesis.* 2008;11(4):381-93
4. Andersen CS, Agger EM, Rosenkrands I, Gomes JM, Bhowruth V, Gibson KJC, Petersen RV, Minnikin DE, Besra GS, **Andersen P**. A simple mycobacterial monomycoated glycerol lipid has potent immunostimulatory activity. *J Immunol.* 2009 Jan 1;182(1):424-432
5. Kamath AT, Rochat AF, Valenti MP, Agger EM, Lingnau K, **Andersen P**, Lambert PH, Siegrist CA. Adult-like Anti-Mycobacterial T Cell and in vivo Dendritic Cell Responses following Neonatal Immunization with Ag85B-ESAT-6 in the IC31® Adjuvant. *PLoS ONE.* 2008;3(11):e3683
6. Ciabattini A, Pettini E, **Andersen P**, Pozzi G, Medaglini D. Primary activation of antigen-specific naïve CD4+ and CD8+ T cells following intranasal vaccination with recombinant bacteria. *Infect Immun.* 2008 Dec;76(12):5817-25
7. Christensen D, Allesø M, Rosenkrands I, Rantanen J, Foged C, Agger EM, **Andersen P**, Nielsen HM. NIR transmission spectroscopy for rapid determination of lipid and lyoprotector content in liposomal vaccine adjuvant system CAF01. *Eur J Pharm Biopharm.* 2008 Nov;70(3):914-20.
8. Wassie L, Demissie A, Aseffa A, Abebe M, Yamuah L, Tilahun H, Petros B, Rook G, Zumla A, **Andersen P**, Doherty TM; for the VACSEL Study Group. Ex Vivo Cytokine mRNA Levels Correlate with Changing Clinical Status of Ethiopian TB Patients and their Contacts Over Time. *PLoS ONE* 3(1): e1522.
9. Rosenkrands I, Aagaard C, Weldingh K, Brock I, Dziegiel MH, Singh M, Hoff S, Ravn P, **Andersen P**. Identification of Rv0222 from RD4 as a novel serodiagnostic target for tuberculosis. *Tuberculosis (Edinb).* 2008 Jul;88(4):335-43
10. Agger EM, Rosenkrands I, Hansen J, Brahimi K, Vandahl BS, Aagaard C, Werninghaus K, Kirschning C, Lang R, Christensen D, Thorsen H, Falch JA, ...

- for (CAF01); a versatile adjuvant for vaccines with different immunological requirements. *PLoS ONE*. 2008 Sep 8;3(9):e3116
11. Kirby DJ, Rosenkrands I, Agger EM, Andersen P, Coombes AG, Perrie Y. Liposomes act as stronger sub-unit vaccine adjuvants when compared to microspheres. *J Drug Target*. 2008 Aug;16(7):543-54.
  12. Hansen J, Jensen KT, Follmann F, Agger EM, Theisen M, Andersen P. Liposome delivery of *C. muridarum* MOMP primes a Th1 response that protect against genital Chlamydia infection in a mouse model. *J Infect Dis*. 2008 Sep 1;198(5):758-67.
  13. Christensen D, Kirby D, Foged C, Agger EM, Andersen P, Perrie Y, Nielsen HM. alpha, alpha'-trehalose 6,6'-dibehenate in non-phospholipid-based liposomes enables direct interaction with trehalose, offering stability during freeze-drying. *Biochim Biophys Acta*. 2008 May;1778(5):1365-1373.
  14. Arend SM, Franken WP, Aggerbeck H, Prins C, van Dissel JT, Thierry-Carstensen B, Tingskov PN, Weldingh K, Andersen P. Double-blind randomized Phase I study comparing rdESAT-6 to tuberculin as skin test reagent in the diagnosis of tuberculosis infection. *Tuberculosis (Edinb)*. 2008 May;88(3):249-61.
  15. Agger EM, Cassidy JP, Brady J, Korsholm KS, Vingsbo-Lundberg C, Andersen P. Adjuvant modulation of the cytokine balance in *Mycobacterium tuberculosis* subunit vaccines: immunity, pathology and protection. *Immunology*. 2008 Jun;124(2):175-85
  16. Weldingh K, Andersen P. ESAT-6/CFP10 skin test predicts disease in *M. tuberculosis*-infected guinea pigs. *PLoS ONE*. 2008 Apr 23;3(4):e1978.
  17. Kamath AT, Valenti MP, Rochat AF, Agger EM, Lingnau K, von Gabain A, Andersen P, Lambert PH, Siegrist CA. Protective anti-mycobacterial T cell responses through exquisite in vivo activation of vaccine-targeted dendritic cells. *Eur J Immunol*. 2008 May;38(5):1247-56
  18. Kirby DJ, Rosenkrands I, Agger EM, Andersen P, Coombes AG, Perrie Y. PLGA microspheres for the delivery of a novel subunit TB vaccine. *J Drug Target*. 2008 May;16(4):282-93.
  19. Follmann F, Olsen AW, Jensen KT, Hansen PR, Andersen P, Theisen M. Antigenic profiling of a *Chlamydia trachomatis* gene-expression library. *J Infect Dis*. 2008 Mar 15;197(6):897-905
  20. Andersen P, Kaufmann SHE. Novel Vaccination Strategies against Tuberculosis, Chapter 7.1 for Volume 2, *Handbook of Tuberculosis, Immunology and Cell Biology*, 1. Edition - January 2008, ISBN-13: 978-3-527-31887-2 - Wiley-VCH, Weinheim
  21. Olsen AW, Follmann F, Højrup P, Leah R, Sand C, Andersen P, Theisen M. Identification of human T-cell targets recognized during the *Chlamydia trachomatis* genital infection. *J Infect Dis*. 2007 Nov 15;196(10):1546-52

- in Denmark detected by M. tuberculosis specific IFN-gamma whole-blood test. *Scand J Infect Dis.* 2007;39(6-7):554-9.
23. Christensen D, Korsholm KS, Rosenkrands I, Lindenstrom T, **Andersen P**, Agger EM. Cationic liposomes as vaccine adjuvants. *Expert Rev Vaccines.* 2007 Oct;6(5):785-796.
  24. **Andersen P.** Tuberculosis vaccines – an update, *Microbiologist,* 2007 Sep;8(73):36-9
  25. Billeskov R, Vingsbo-Lundberg C, **Andersen P**, Dietrich J. Induction of CD8 T Cells against a Novel Epitope in TB10.4: Correlation with Mycobacterial Virulence and the Presence of a Functional Region of Difference-1. *J Immunol.* 2007 Sep 15;179(6):3973-81
  26. Lyashchenko KP, Greenwald R, Esfandiari J, Greenwald D, Nacy CA, Gibson S, Didier PJ, Washington M, Szczepański P, Motzel S, Handt L, Pollock JM, McNair J, **Andersen P**, Langemans JA, Verreck F, Ervin S, Ervin F, McCombs C. PrimaTB STAT-PAK(R) Assay, a Novel Rapid Lateral-Flow Test for Tuberculosis in Non-human Primates. *Clin Vaccine Immunol.* 2007 Sep;14(9):1158-64.
  27. Hoff ST, Abebe M, Ravn P, Range NS, Malenganisho WL, Rodrigues DS, Kallas EG, Søborg C, Doherty TM, **Andersen P**, Weldingh K. Evaluation of Mycobacterium tuberculosis-specific Antibody responses in populations with different levels of exposure from Tanzania, Ethiopia, Brazil and Denmark, *Clin Infect Dis.* 2007 Sep 1;45(5):575-82
  28. **Andersen P.** Tuberculosis vaccines – an update, *Nature Reviews Microbiology* 2007 Jul;5(7):484-7
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